Clinical and Experimental Pharmacology and Physiology (2004) **31**, 580–590

EFFECTS OF INACTIVATION OF THE LATERAL PULVINAR ON RESPONSE PROPERTIES OF SECOND VISUAL AREA CELLS IN *CEBUS* MONKEYS

JGM Soares, ACM Diogo, M Fiorani, APB Souza and R Gattass

Laboratory de Fisiologia da Cognição, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

SUMMARY

1. In the present study, we investigated the influence of the pulvinar nucleus upon response properties of single cells in the second visual area (V2) of *Cebus* monkeys. The method used consisted of the inactivation of a portion of the lateral pulvinar by GABA injections while studying the response properties of cells in V2 at the same visuotopic location as that of the inactivation.

2. After GABA injection in the pulvinar, most cells in V2 (67%) showed changes in spontaneous and/or stimulus-driven activities. Contrary to the effect found with inactivation of the striate cortex, which promotes a reduction in the response of V2 neurons, we found that the main effect of pulvinar inactivation was an increment in stimulus-driven responses of V2 cells (39% of units studied). A reduction of responses was observed in 27% of units.

3. A change in orientation and/or direction selectivity was found in 91% of cells after inactivation of the pulvinar. Most commonly, the orientation selectivity of a neuron was decreased during pulvinar inactivation.

4. The inactivation results indicate that the pulvinar projections have a modulatory effect on the activity of V2 cells.

Key words: orientation selectivity, posterior thalamus, prestriate cortex, primate, visual system.

INTRODUCTION

The pulvinar nucleus is a diencephalic structure located in the posterior region of the thalamus whose evolutionary development occurred in parallel with the expansion and differentiation of the temporo–parieto–occipital cortex.[1] The involvement of the pulvinar nucleus with visual function has been demonstrated by the presence of a retinotopic organization[2–4] and by its connections with different cortical visual areas.[5–10] In cats, neurons in cortical areas 17 and 18 are affected during lateral posterior–pulvinar complex inactivation, showing modulation of the strength of the oscillations evoked by visual stimuli.[11] However, the function of the massive pulvino–cortical projections in primates remains unknown. Although the pulvinar has been implicated in conveying visual information to the extrastriate cortex in monkeys with striate cortex (V1) lesions,[12,13] there are few data on its participation in the normal physiology of visual perception.

Several studies have suggested the involvement of the pulvinar in mechanisms of visual attention. In monkeys, the deactivation of a portion of the pulvinar with muscimol injections caused delay on attentional tasks.[14] Deficits in visual attention were also found in patients with thalamic lesions involving the pulvinar nucleus. These patients showed a specific deficit in the ability to use attention to improve the efficiency of processing visual targets contralateral to the lesion.[15] In addition, a study with positron emission tomography showed a greater activity of the pulvinar while the subject performed an object-identification task, which required attentional selection.[16]

In primates, the second visual area (V2) is the largest extrastriate area and receives massive projections from the pulvinar,[7,9,17] mainly from its lateral portion.[9] Nonetheless, inactivation of V1 renders V2 neurons completely silent,[18,19] raising the question of the role of the pulvinar projections in shaping the response of V2 cells. In the present study, we have used a technique of temporary inactivation with injections of GABA to examine the role of the pulvinar nucleus upon the activity of single cells in visual area V2. We have demonstrated an overall change in the response to visual stimuli, as well as in the orientation/direction selectivity of cells recorded in area V2 during pulvinar inactivation. A preliminary account of these data has been presented elsewhere.[20]

METHODS

Two adult *Cebus apella* monkeys were used in chronic preparations in once-weekly recording session for a total of 20 sessions. All experimental protocols were conducted following National Institutes of Health (NIH) guidelines for animal research and were approved by the Committee for Animal Care and Use of the Instituto de Biofísica Carlos Chagas Filho, UFRJ. Prior to the first recording session, a bolt for holding the head of the animal in a stereotaxic apparatus and a recording chamber were implanted in the skull under anaesthesia and aseptic conditions. The maintenance and monitoring of the animal during recording sessions have been described in detail previously.[21] Briefly, on each recording day, anaesthesia was induced with ketamine hydrochloride (30 mg/kg, i.m.) and maintained with a mixture of 70% nitrous oxide and 30% oxygen, combined with a continuous intravenous infusion of fentanyl citrate (0.03 mg/kg per h). Animals were also immobilized with pancuronium bromide (0.1 mg/kg per h). Body temperature, EKG and end-tidal CO_2 were monitored continuously.

Correspondence: Ricardo Gattass, Programa de Neurobiologia, Instituto de Biofísica Carlos Chagas Filho, Bloco G, CCS, UFRJ, Ilha do Fundão, Rio de Janeiro, RJ 21941-900, Brazil. Email: rgattass@biof.ufrj.br

Received 26 February 2004; revision 28 May 2004; accepted 3 June 2004.

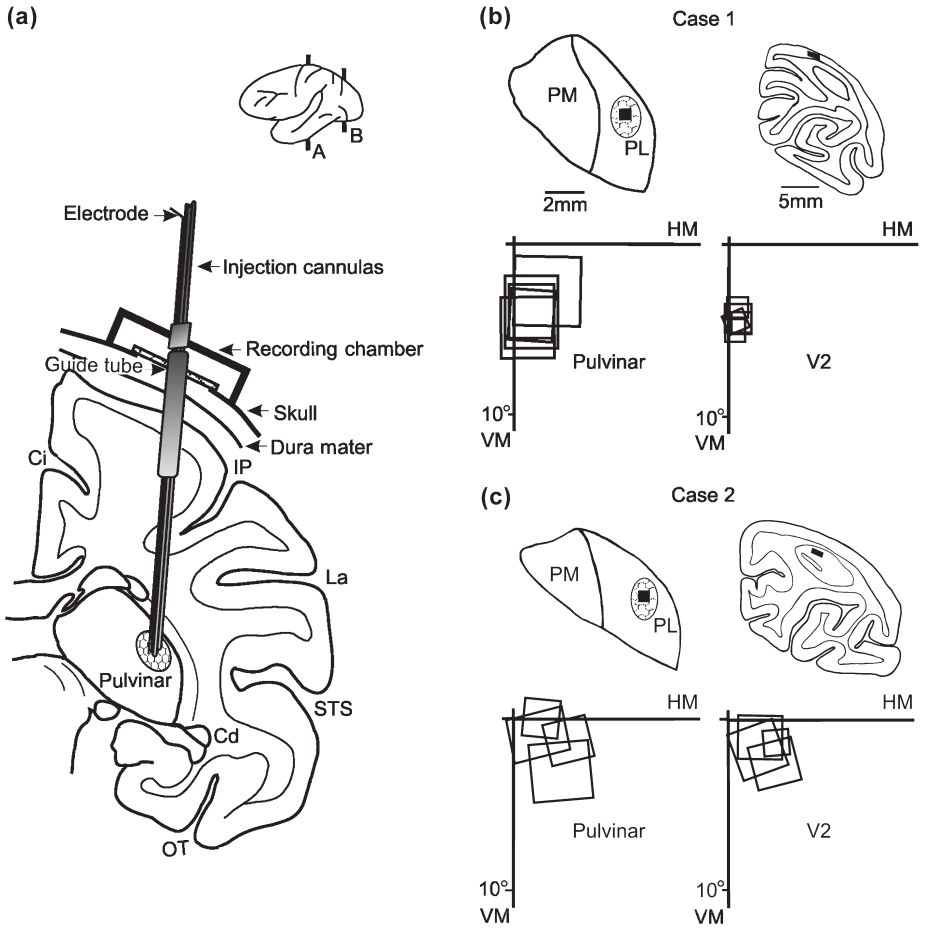

Fig. 1 Injection site in pulvinar and receptive fields of cells in pulvinar and V2. (a) Schematic drawing of a coronal section of the brain of a *Cebus* monkey, at the level A illustrated in the inset, showing the assembly of electrode and injection cannulas and the approximate extent of the inactivated region in the pulvinar in case 1. (b) Coronal section of the pulvinar (left) at the level A in the inset showing the location of recording (black squares) and injections sites (hatched ovals) in the pulvinar and a section at level B (right) showing the location of recording sites in V2 (black squares). Lower, receptive fields of cells recorded at the injection site in the pulvinar and at the corresponding topographical locations in area V2 in case 1. (c) Same as (b) for case 2. Cd, caldate nucleus; Ci, cingulate sulcus; HM, horizontal meridian; IP, intraparietal sulcus; La, lateral sulcus; PL, lateral pulvinar; PM, medial pulvinar; OT, occipitotemporal sulcus; STS, superior temporal sulcus; VM, vertical meridian.

To locate the pulvinar nucleus, we made penetrations with single tungsten microelectrodes, through a guide tube, using the stereotaxic coordinates proposed by Gattass *et al.*[4] After localization of the lateral pulvinar, the guide tube was fixed to the skull to allow access to the same region of the nucleus in the following recording sessions.

At each experimental session, prior to inactivation, we mapped the receptive fields at corresponding topographical locations in the pulvinar and in area V2. Repeated cycles of inactivation and recording are known to result in a gradual deterioration of the quality of recordings.[22] When such activity was no longer detected, the experiment was discontinued and the animal was killed for histological processing for the localization of injection and recording sites.

GABA injections were made under electrophysiological control using a tungsten microelectrode, 0.5 MΩ impedance, attached to an assembly of three stainless-steel tubes (cannulas) 300 μm in diameter, glued at different heights at 500 μm intervals. The GABA injections (0.5 mol/L, 1–2 μL in each cannula) were made using a pneumatic pump (Pneumatic Pico-Pump model PV820; World Precision Instrument, Sarasota, FL, USA). Figure 1a illustrates a coronal section of a cerebral hemisphere showing the assembly of electrode and injection cannulas and the approximate extent of the inactivated region of the pulvinar in case 1. The local effect of GABA in the pulvinar was evaluated by means of electrophysiological recording at the injection site.

Single units in V2, recorded at the same visuotopic location as that of the pulvinar (Fig. 1b,c), were studied using the program CORTEX (NIH, Bethesda, MD, USA). The visual stimuli, presented on a computer monitor placed at a distance of 57 cm in front of the animal, consisted of a thin white bar (18°× 0.5°) that appeared in four random orientations and crossed the screen in a direction perpendicular to its orientation, with a velocity of 6°/s, passing through the receptive field. Each trial consisted of 10 presentations of the bar in each of the eight directions of movement. The

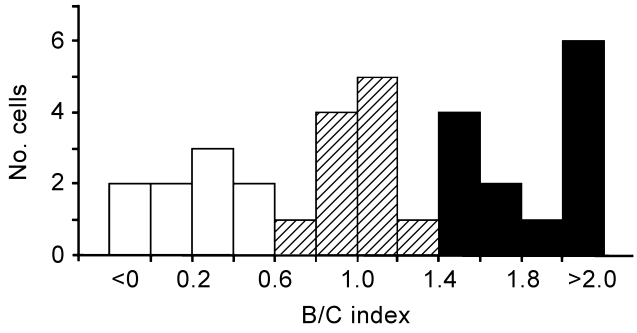

Fig. 2 Distribution of the blocked/control (B/C) index of V2 cells. (□), cells (27.3%) with a significant decrease of the response; (▨), cells (23.3%) with no significant change in response; (■), cells (39.4%) with a significant increase of the response during pulvinar inactivation. (See text for details.)

response of cells to the visual stimulus was studied before and after GABA injections.

Pharmacological inactivation paradigms are based on postinjection effects that disappear over time. Usually the effects are reversible and they are evaluated in comparison with the pre-injection state. The return to the resting or pre-injection spontaneous and driven activity is assurance of a non-toxic or non-destructive effect on the central nervous tissue. In our experiment, we use a paradigm aiming at inactivation of a large portion of the lateral pulvinar, which has reciprocal connections with V2. The mechanical insertion of the cannula in a small region of the pulvinar and the large amount of GABA injected over many experimental sessions caused minor damage detected after histological processing. Inasmuch as driven cellular activity was detected prior to inactivation in all recording sessions, we

assume that the functional effect of tissue damage was negligible at the time of recording. In addition, we studied the time-course of changes in V2 after pulvinar inactivation to rule out destructive effects of the mechanical or pharmacological approaches. When we started these experiments, we had no previous knowledge of the long-lasting effect caused by such large injections. Therefore, we based our results on the sample of cells in which a clear trend towards recovery of the driven activity was observed.

Extracellular single unit activity from area V2 was recorded using tungsten microelectrodes (0.5 MΩ impedance). Cellular activity was amplified, filtered, isolated by means of a waveform discriminator system (SPS-8701; Signal Processing Systems, Aston, UK) and the spike time was stored by the CORTEX program (Laboratory of Neuropsychology, NIMH/NIH, Bethesda, MD, USA) for off-line analysis. Peristimulus time histograms (PSTH) were computed for 10 presentations for each direction of motion (bin width of 10 msec, smoothing by a Gaussian filter of 200 msec). After a correction for latencies and the spread of the neuronal responses throughout the stimulus length in the proper orientation, the PSTH for each axis of movement was converted into a two-dimensional distribution of the spike density in spatial coordinates (orientation response map). The average orientation response maps for all tested orientations results in a qualitative intersection map that shows the location of the receptive field.[23] The response of the cell (spike/s) in each PSTH at all times before and after the injection was computed within the same time window that comprises the peak firing rate of the qualitative intersection map. Spontaneous activity was calculated as the average firing rate in the 700 msec preceding stimulus motion in all trials.

Student's *t*-test was used to test the significance of the response to moving bars by comparing the response of the cell with the previous spontaneous activity. For the calculation of all indices, the value of the spontaneous activity was subtracted from that of the response. We used a blocked/control (B/C) index to quantify the changes in relative responsiveness as a result of GABA injections. The B/C index was calculated as R_B/R_C, where R_C is the response in the preferred direction before GABA injection and R_B is the response in the same direction after the injection. We considered that a change in cell activity occurred when its B/C index was less than 0.6 or greater than 1.4.[22] One-way ANOVA was used to verify whether the response depended on the orientation/direction of the stimuli. Orientation selectivity was quantified by the orientation index (OI) calculated as $OI = 1 - R_{ort}/R_{max}$, where R_{max} is the response in the preferred orientation and R_{ort} is the response in the orthogonal orientation. The quantification of direction selectivity was achieved by the directional index (DI), which compares responses to opposite directions of motion:

Table 1 Summary of the effects of GABA injection in the pulvinar nucleus over V2 cells

		Change in modulation			Change in selectivity			
	Total	Excitatory modulation	Inhibitory modulation	No change	Change OI	Change DI	Change OI and DI	No change
No. cells (%)	33	13 (39.4)	9 (27.3)	11 (33.3)	7 (21.2)	5 (15.2)	18 (54.5)	3 (9.1)

OI, orientation index; DI, directional index.

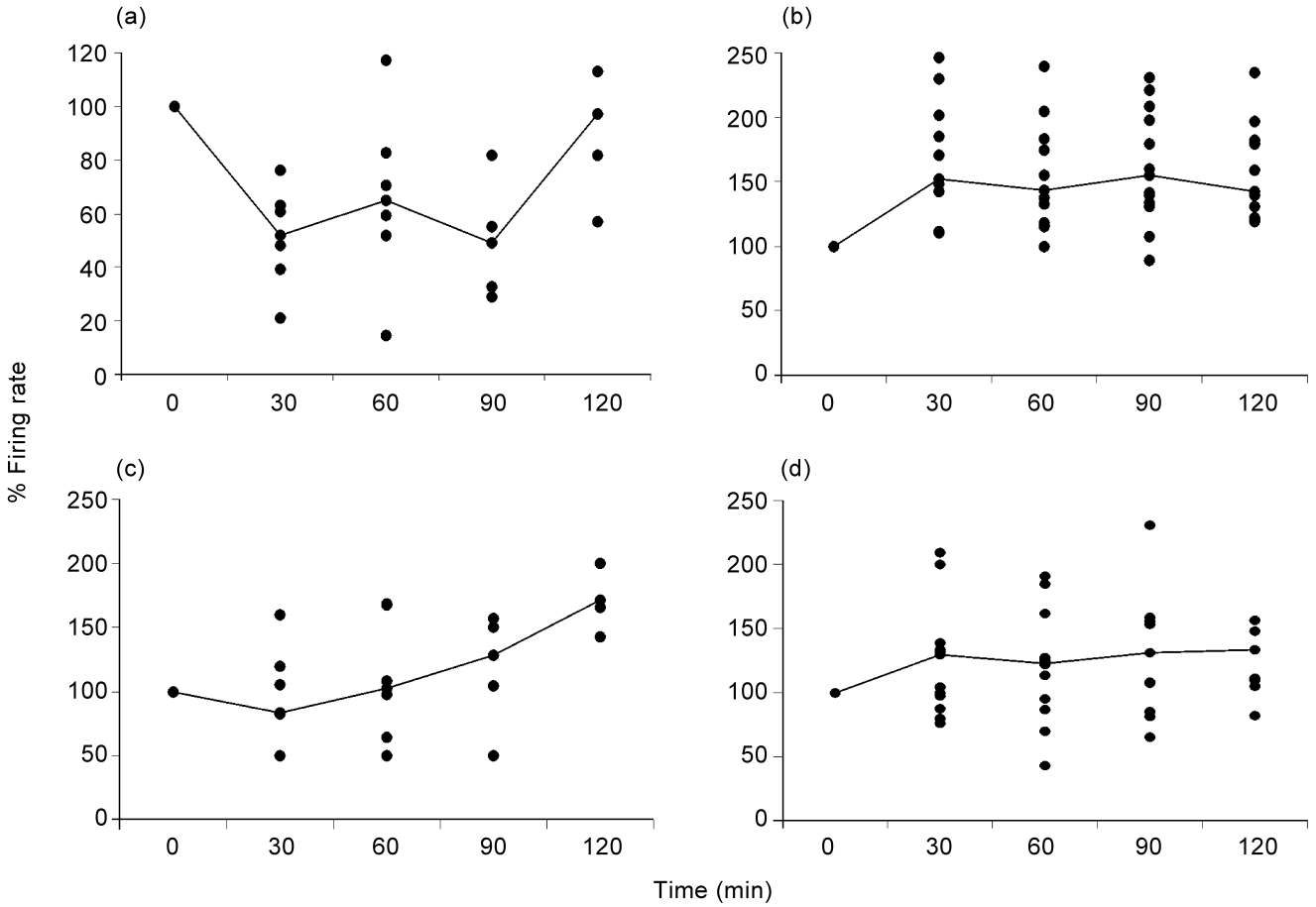

Fig. 3 Time-course of the cell response (a,b) and of spontaneous activity (c,d) for the inhibitory (a,c) and excitatory (b,d) modulation of cell groups relative to the activity prior to injection. At each time, the dots represent the relative firing rate for each cell. The line represents the time-course of the median.

$DI = 1 - R_{min}/R_{max}$, where R_{max} and R_{min} are responses in the preferred direction and a diametrically opposed direction, respectively. We considered a change in OI or DI greater than 0.20 as significant.[24]

To control for the non-specific effects of injections of large volumes of GABA, we injected equal amounts of GABA into a temporal area close to the pulvinar. No effect on the properties of V2 cells was found after injections of saline or GABA into this temporal cortical region close to the pulvinar.

After the last recording session, animals were deeply anaesthetized with sodium pentobarbitone (30 mg/kg) and perfused intracardially with normal saline followed by 2% paraformaldehyde in phosphate-buffered saline (PBS); 2% paraformaldehyde in PBS + 2.5% glycerol; PBS + 5% glycerol; and PBS + 10% glycerol. Serial 40 μm coronal sections were obtained using a cryostat. Adjacent series were stained for cell bodies with cresyl violet and for fibres with Gallyas' stain to allow reconstructions of the electrode tracks and to define the borders of V2 as well as those of the pulvinar nucleus.

RESULTS

In the pulvinar nucleus, GABA injections (1 μL in each of the three cannulas) resulted in a significant decrease of cellular activity and, 5 min after the injection, the activity was, on average, 40% that of the initial level. Unexpectedly, recovery of activity at the injection site was rather slow, taking more than 70 min to return to pre-injection levels. This suggests that the mechanical stress produced by pressure injections may have contributed to the local inactivation or that injections of large amounts of GABA may result in longer-term effects.

We studied 33 cells in V2 before and after GABA injections in the pulvinar. All cells had their receptive fields within 10° of the representation of the central visual field (Fig. 1b,c). Most cells studied in V2 (67%) during pulvinar inactivation showed changes in the response to visual stimuli and/or in spontaneous activity. Figure 2 shows changes in the response of cells quantified by the B/C index. Of these cells, 39% showed an increase, whereas 27% showed a decrease of the response. We classified the cells as: (i) cells with excitatory modulation when they showed an immediate increase of the cell response after inactivation; and (ii) cells with inhibitory modulation when they showed an immediate decrease of the cell response after GABA injection in the pulvinar. Although the paradigm used to study direction/orientation selectivity included only eight directions, we observed a change in the direction and/or orientation selectivity in 91% of cells during

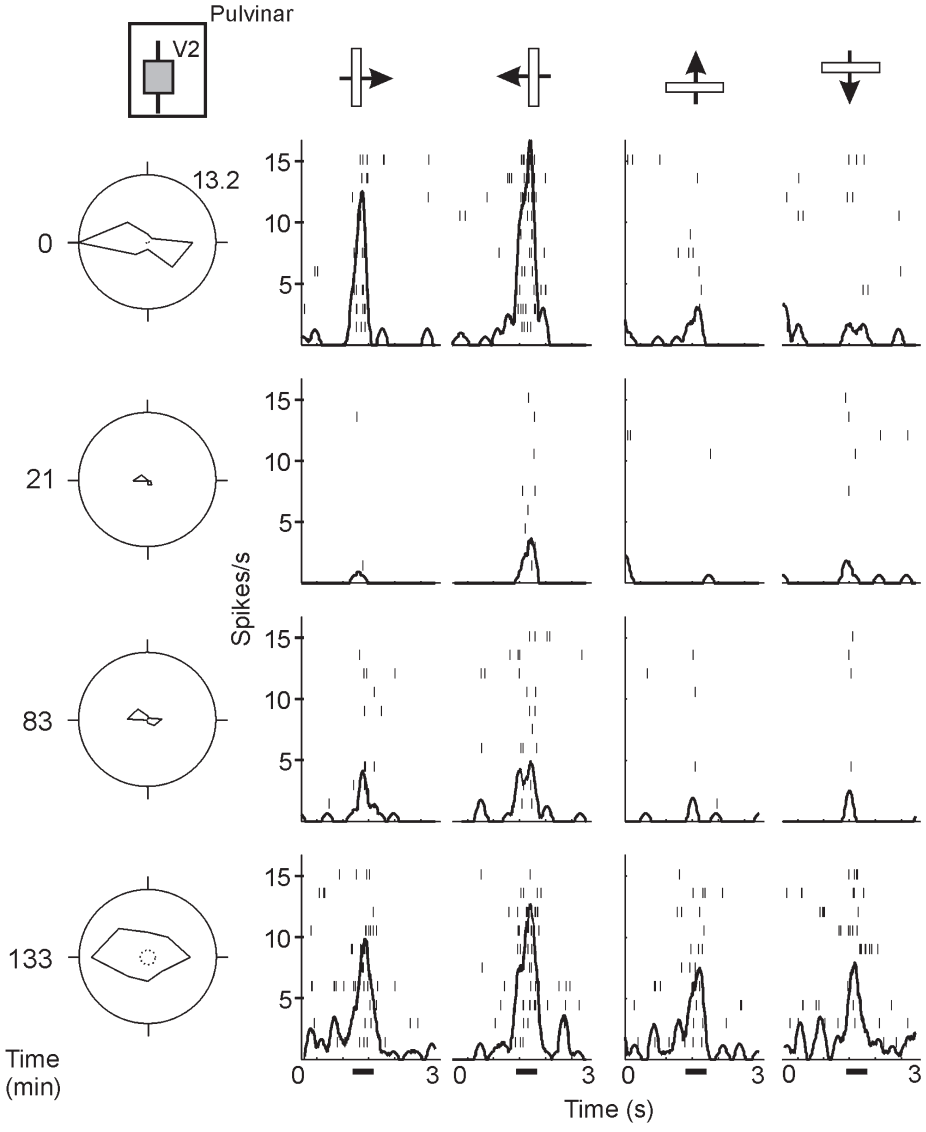

Fig. 4 Overall decrease of the visual responsiveness of a V2 cell after GABA injection. Cell activity was recorded before (0) and at different time intervals (21, 83 and 133 min) after injection of GABA. The inset shows the receptive field of the cell of V2 relative to the receptive field recorded at injection site in the pulvinar. Middle columns: peristimulus time histograms (PSTH) of the response to stimulus movement in the preferred direction (180°) and in the opposite direction. Right columns: PSTH of the response to stimulus movement in orthogonal directions. Vertical ticks represent spikes in 10 trials. Left columns: polar diagrams displaying the mean response rates computed from the regions corresponding to the receptive fields (dark bars below PSTH) for different directions of movement, at 45° steps. Dotted-line circles in the centre of the polar diagrams correspond to the mean spontaneous activity of the cell. The radii of the external circles indicate the maximum value of the cell's response (13.2 spikes/s) obtained throughout recording. Note the large reduction of the cell response, which starts to recover 83 min after the injection.

pulvinar inactivation. Most of these cells (55%) showed changes in both DI and OI, whereas 15% showed changes only in DI and 21% showed changes only in OI. Table 1 summarizes the changes that occurred in V2 cells after GABA injections in the pulvinar nucleus.

Cells with inhibitory modulation

Of the 33 cells studied in V2, nine (27%) showed a decrease of the cell response and/or spontaneous activity after injection of GABA in the pulvinar. Seven of these cells showed a decrease in the response to the moving bar after inactivation, with a late recovery. In addition, in most cells the spontaneous activity increased as an after inactivation rebound. Figure 3a,c shows the time-course of the effects of the pulvinar inactivation over these V2 cells. An example of this type of inhibitory modulation is illustrated in Fig. 4. This cell presented a long-lasting strong reduction of its response after inactivation with minor effects on spontaneous activity. The recovery of the response occurred only 130 min after injection.

The remaining two cells showed a different behaviour, exhibiting an inhibitory receptive field during the inactivation of the pulvinar. These cells have properties of GABAergic interneurons. They showed high spontaneous activity and poor orientation selectivity. Figure 5 illustrates one of these cells, which changed the type of

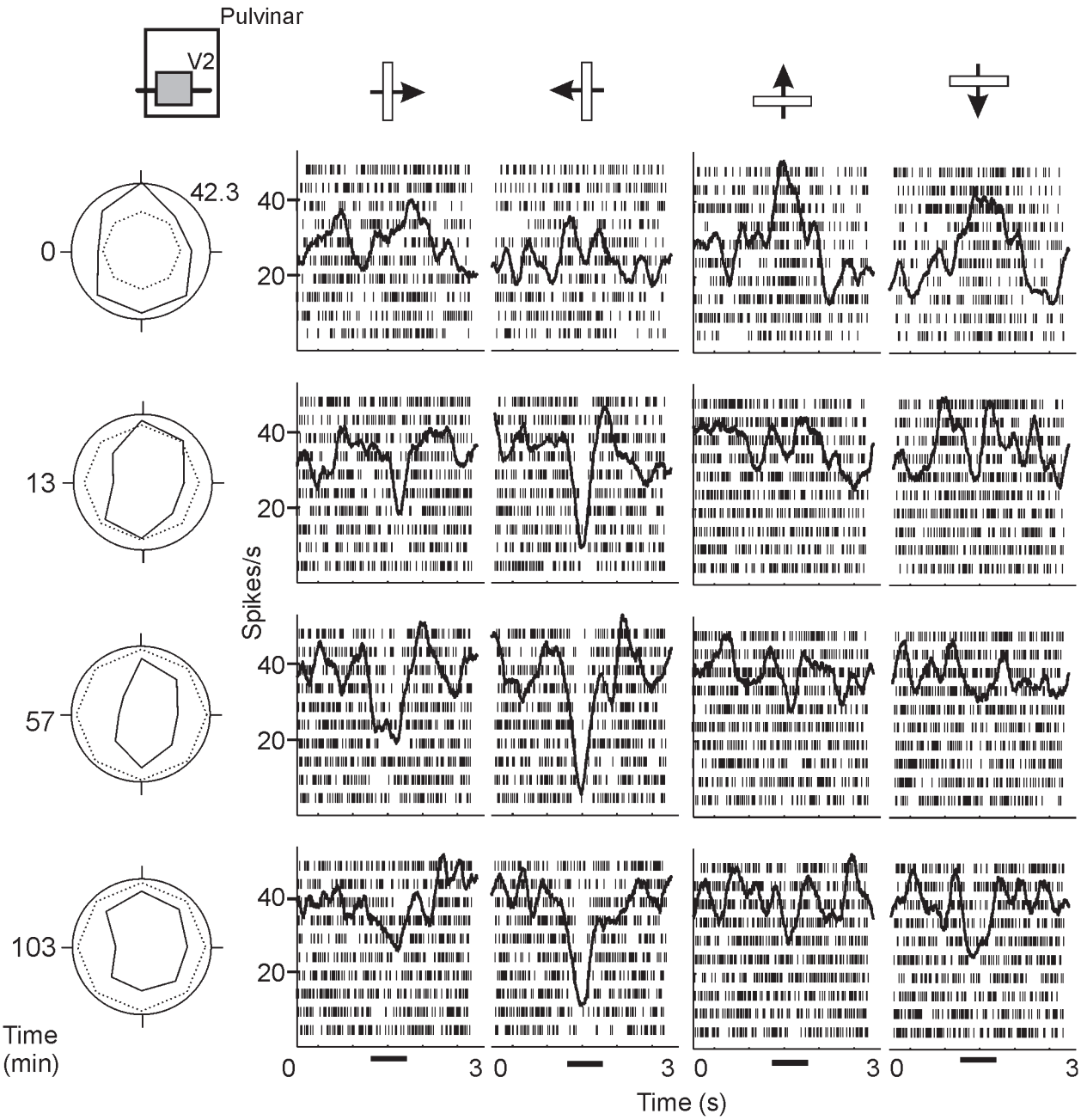

Fig. 5 Change of response type of a V2 cell during inactivation of the pulvinar. Prior to inactivation, this cell showed an excitatory receptive field with a better response for movements along the 90–270° axis. After GABA injection, the spontaneous activity increased and the cell started to exhibit an inhibitory receptive field. (See also legend for Fig. 4.)

response after inactivation of the pulvinar. Prior to inactivation, this cell showed an excitatory receptive field with a better response for movements along the 90–270° axis. After inactivation, the cell started to exhibit an inhibitory receptive field, concomitant with an increase in spontaneous activity.

Cells with excitatory modulation

After GABA injections in the pulvinar, we observed an increase in the spontaneous activity and/or in the response to visual stimuli in 13 (39%) of cells studied in V2. The majority of cells showed a late partial recovery of the response after GABA inactivation. As observed in cells with inhibitory modulation, these cells also maintain a high spontaneous activity throughout the experiment (Fig. 3b,d). As a result, in some of these cells the direction/orientation selectivity is slightly different from that obtained before the injection.

Figure 6 illustrates a cell that showed an excitatory modulation in both its response to the stimulus as well as spontaneous activity during inactivation of the pulvinar. Before the injection, this cell showed low spontaneous activity and a preferred direction of 180° to a moving bar. After GABA injection, we observed an enhancement of the cell response to the preferred direction, concomitant with a decrease in directionality due to an increase of the response to the opposite direction. After 119 min, there is a reduction in the direction/orientation selectivity. After 149 min, the cell response begins to return to initial values; the spontaneous activity, however, remains increased.

Figure 7 illustrates a cell that initially (14 min) shows an increase in its direction selectivity due to an increase in the

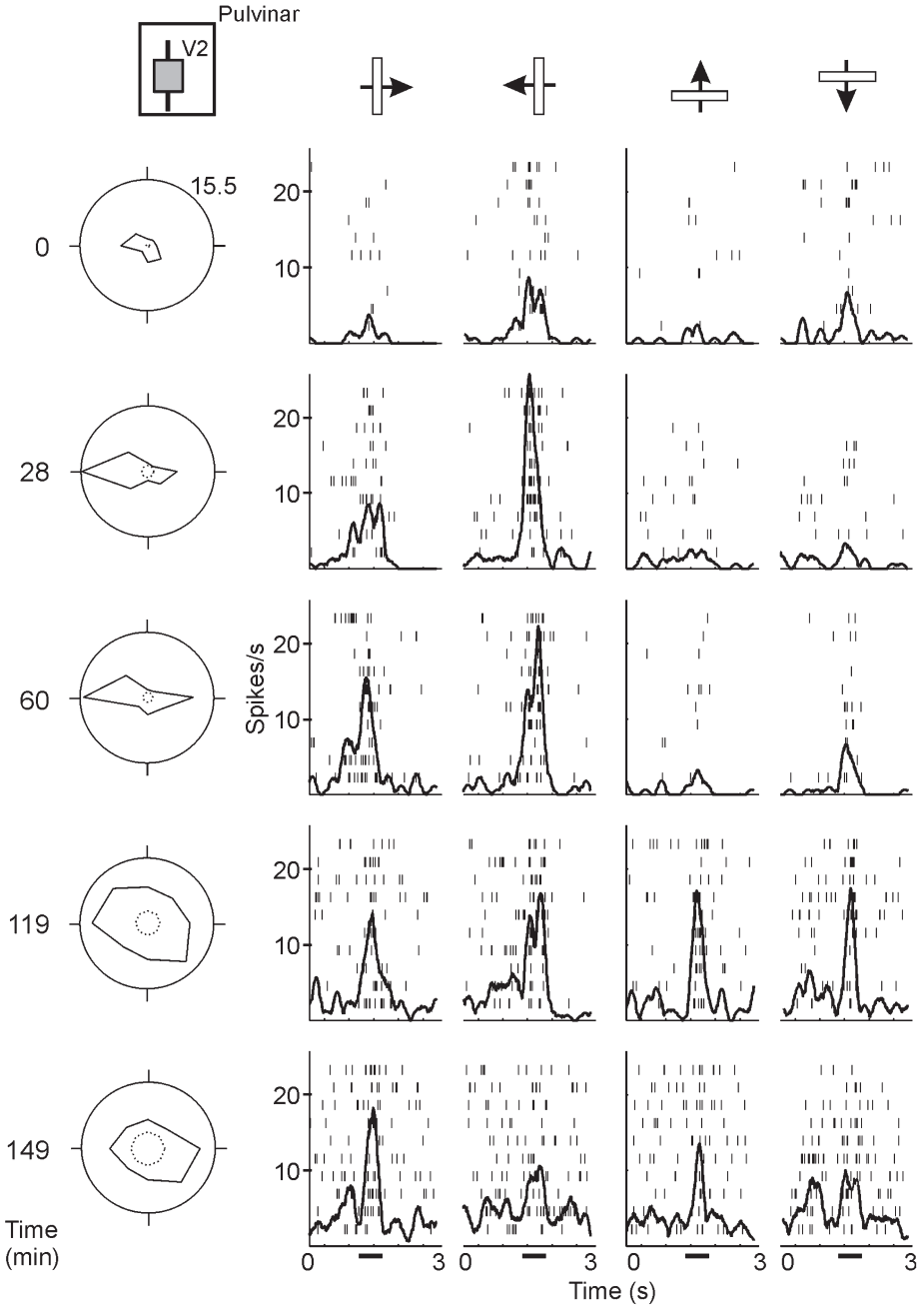

Fig. 6 Increase of the response of a V2 cell with a concomitant decrease in the directional index (DI). In spite of the increase of the cell's response to the preferred direction (180°), the direction selectivity of the cell decreases due to the simultaneous increase of the response in the opposite direction. DI(0) = 0.72; DI(60) = 0.32. (See also legend for Fig. 4.)

response to the moving bar in its preferred direction (45°), as well as to a decrease in the opposite direction. After 60 min, the response begins to return to the initial values and, after 90 min, the cell response is similar to that recorded prior to injection.

The initial change in directionality observed in V2 cells after GABA injections in the pulvinar, such as those illustrated in Figs 6,7, is not observed in all cells. Some cells lose their orientation/direction selectivity and show a concomitant increase in their spontaneous activity. One of these cells is illustrated in Fig. 8. This cell had orientation/direction selectivity with a better response for movements along the 45–225° axis. After inactivation, we observed a decrease of the cell response concomitant with an increase of its spontaneous activity, with a loss of the orientation tuning.

Changes in orientation and direction selectivity

In addition to excitatory and inhibitory modulations in the response, most cells in V2 also showed changes in the orientation and/or direction selectivity after GABA injections. In few cases, the cells showed changes in selectivity in the absence of either excitatory or inhibitory overall modulation. Figure 9 is an example of a cell that shows an increase in both the orientation and directional indices during pulvinar inactivation. This cell had a better response to the direction of 315° and, after GABA injection, showed a decrease of the response to this direction and an increase in the response to the opposite direction.

Figure 10 shows a comparison of DI (Fig. 10a) and OI (Fig. 10b) of cells in V2 before and after GABA injections in the pulvinar. The inactivation of the pulvinar caused a significant change in DI (> 0.2) in 23 of 33 cells (70%) studied in V2. Of these cells, 36% showed an increase in their directionality, whereas 33% showed a decrease. After GABA injections, there is a tendency for cells with a smaller DI (< 0.5) to increase their directionality, whereas those with a greater DI tend to lose their directionality. A large percentage of cells (76%) showed a significant change in their OI. Of these cells, 27% presented an increase in OI, whereas 49% showed a decrease. Most cells with high orientation selectivity (OI > 0.5) tend to reduce the OI after inactivation due to an increase in its orientation bandwidths.

DISCUSSION

After the inactivation of the pulvinar, most cells studied in V2 showed changes in both spontaneous activity and the response to

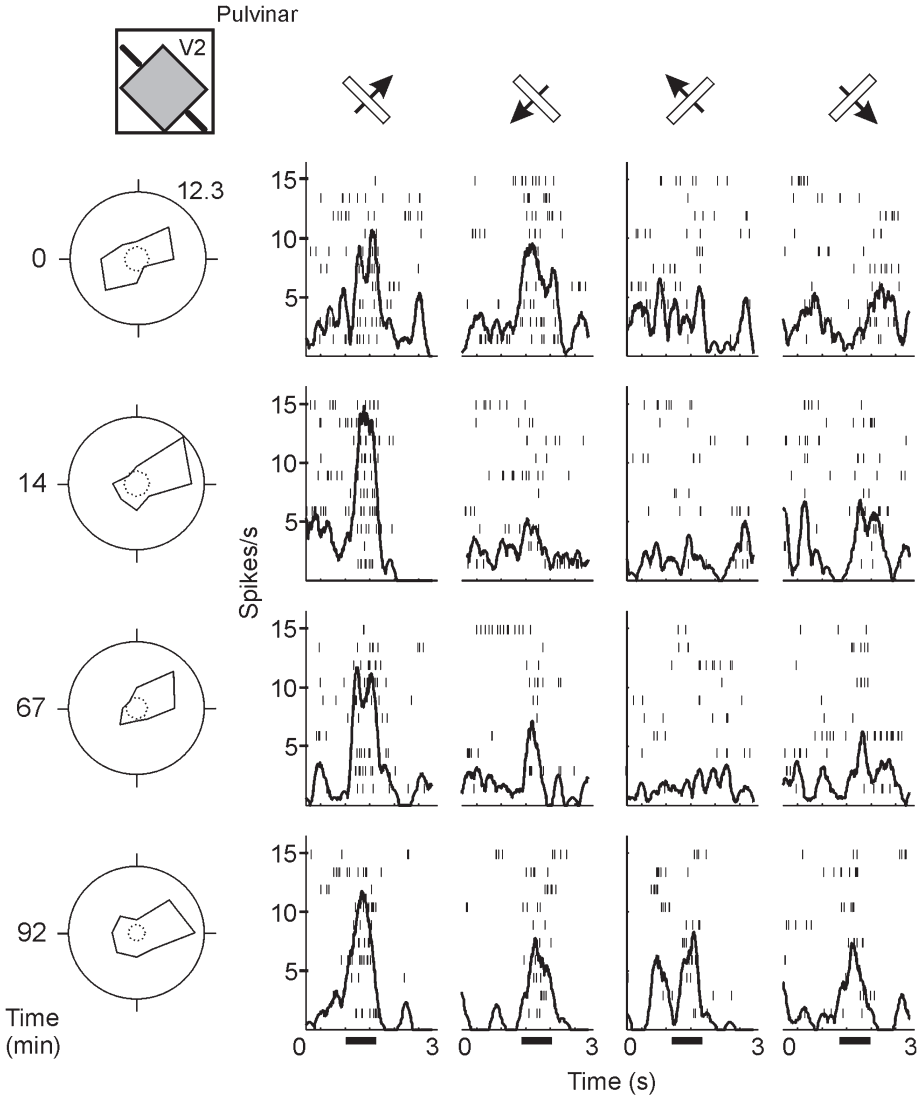

Fig. 7 Increase of the response of a V2 cell with a simultaneous increase in directional index (DI). Note the increase in the direction selectivity due to the increase of the cell's response to the preferred direction (45°) and the decrease to the opposite direction of stimulus movement after GABA injection. DI(0) = 0.03; DI(14min) = 0.83. (See also legend for Fig. 4.)

visual stimuli. A change in orientation and/or direction selectivity was found in 91% of these cells, suggesting that the pulvinar has a modulatory effect over the activity of V2 cells.

In a typical inactivation experiment, one expects to see a transient effect that disappears with time. Several authors have reported short recovery periods after GABA inactivation of the cortex.[24–26] In these experiments, GABA inactivation was achieved by iontophoresis or pulsed injections of very small quantities of GABA and local or short interareal connections were investigated. Others studies[22,27,28] have shown that the duration of inactivation is directly proportional to the volume injected and to the concentration of GABA injected. In addition, these studies have also shown that repeated injections increase the duration of inactivation and the strength of its effect.[22,27,28] In the present study, we made multiple injections of 1–2 μL of 0.5 mol/L GABA, at three different depths, 500 μm apart, to inactivate a large area of the pulvinar. The decrease in cell activity at the site of inactivation after GABA injection was immediate, with a late recovery. The activity returned to its initial value only 70–150 min after injection. This long-lasting action could be due either to the high concentrations of GABA used or to the large volumes injected. The histological processing made after five to eight inactivation sessions showed a lesion in the region of GABA application, which may have been caused by the large volume injected or by repeated injections at the same site. Casanova *et al.* also observed lesions after more than three injections at the same site in the cortex.[22]

The prolonged effect of GABA injections in the pulvinar on the activity of V2 cells could also be explained by an effect of GABA on the local metabolism of cells in the pulvinar. In pulvinar cells, the administration of GABA in high concentrations could reduce the synthesis of endogenous GABA due to an inactivation of the glutamic acid decarboxilase enzyme (GAD). In cultured cells of chicken embryo, the administration of GABA reduces the expression of GAD in a dose-dependent manner.[29] The activity of the GAD is reduced to 50% in the presence of 0.01 mmol/L GABA. After the withdrawal of GABA, the activity of the enzyme takes several hours to return to normal.[29]

Moreover, changes in the pattern of spontaneous activity may also be related to changes in cortical rhythms that may characterize a special operational mode of cortical networks.[30] Thus, it would be very likely indeed that pulvinar projections could change the cortical dynamics, influencing signal processing at the target areas.

Most cells in V2 showed changes in their response to visual stimuli at the beginning of inactivation of the pulvinar. In some cells, there was also a general increase in activity, which usually peaked near 70 min after the injection. The change in directionality of V2 cells, which occurs at the beginning of the inactivation period, could be related to a direct effect of the pulvinar over V2. However, the general increase in cell activity that occurs at approximately 70 min could be explained either by a rebound effect at the end of the inactivation period or by the existence of indirect circuits that involve other areas of the visual cortex that receive connections from the pulvinar and project back to area V2, such as areas V4, MT and the inferotemporal cortex.[31–33]

GABA can act over several structures that constitute the complex synaptic glomeruli of the pulvinar. The action of GABA onto these synapses could reduce the depolarization of post-synaptic cells that project to the cortex. In the case of the excitatory pulvinar

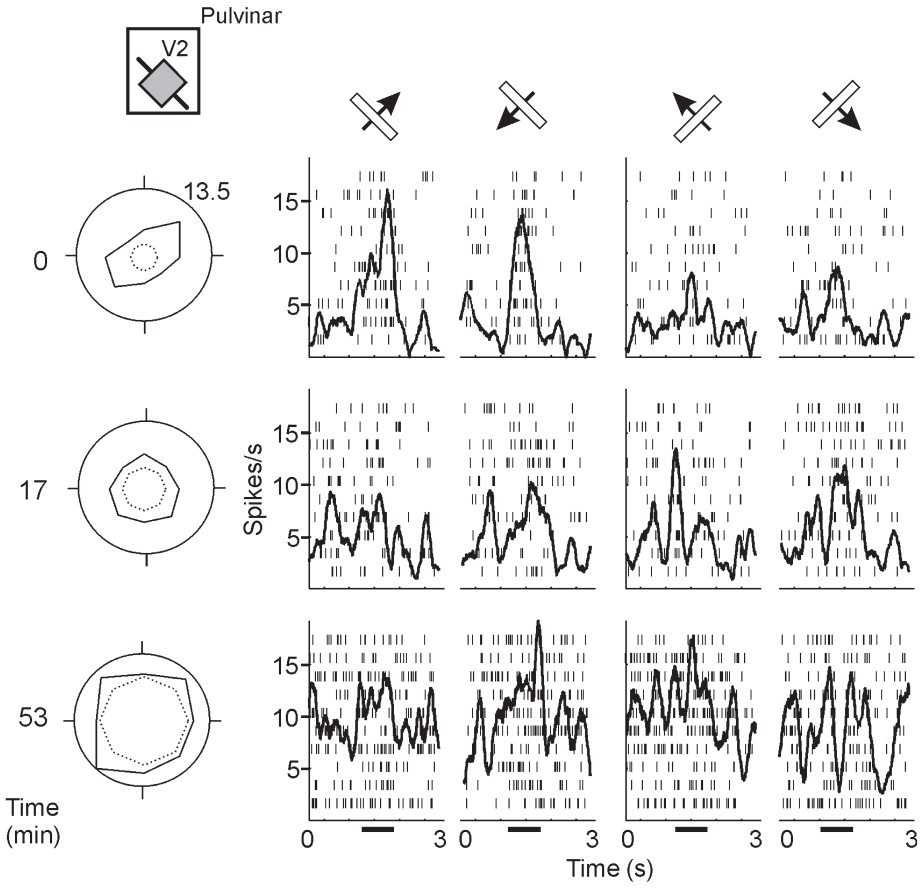

Fig. 8 Loss of orientation/direction selectivity of a V2 cell. This cell had a better response for movements along the 45–225° axis. After GABA injection, there is a clear reduction in the cell's response concomitant with an increase in spontaneous activity, paralleled by a decrease of the orientation index (OI). OI(0) = 0.73; OI(17min) = 0.11. (See also legend for Fig. 4.)

projections to V2, GABA injection could cause the cells of V2 to lose this direct excitatory modulation. Alternatively, the pulvinar projections could have an inhibitory effect by activating inhibitory interneurons in V2, similar to the cell illustrated in Fig. 5. The fact that the majority of V2 cells show an increase in the response to visual stimuli during inactivation of the pulvinar suggests that inhibitory interactions are an important part of the effect of the pulvinar on V2.

These data support the notion of the pulvinar as a transthalamic corticocortical modulator[34] inasmuch as its inactivation does not silence the target neurons. However, the inactivation of the pulvinar modulates receptive field properties, such as direction/orientation selectivity of V2 neurons.

The pulvinar provides the major subcortical input to V2. Pulvinar terminal zones align with regions of increased cytochrome oxidase (CO) staining, avoiding the pale stripes.[35] All V2 stripes receive input from V1; however, the heaviest inputs are to the pale CO stripes. This suggests that inputs from V1 and from the pulvinar may target distinct populations of neurons in V2.[36] In the present study, we did not investigate the location of the cells relative to the cytochrome oxidase modules in area V2; however, the different effects of pulvinar inactivation observed could reflect the fact that these cells could be located in different modules of V2. A study of the selectivity of cells in the different CO stripes after inactivation of the pulvinar would be necessary to address this question.

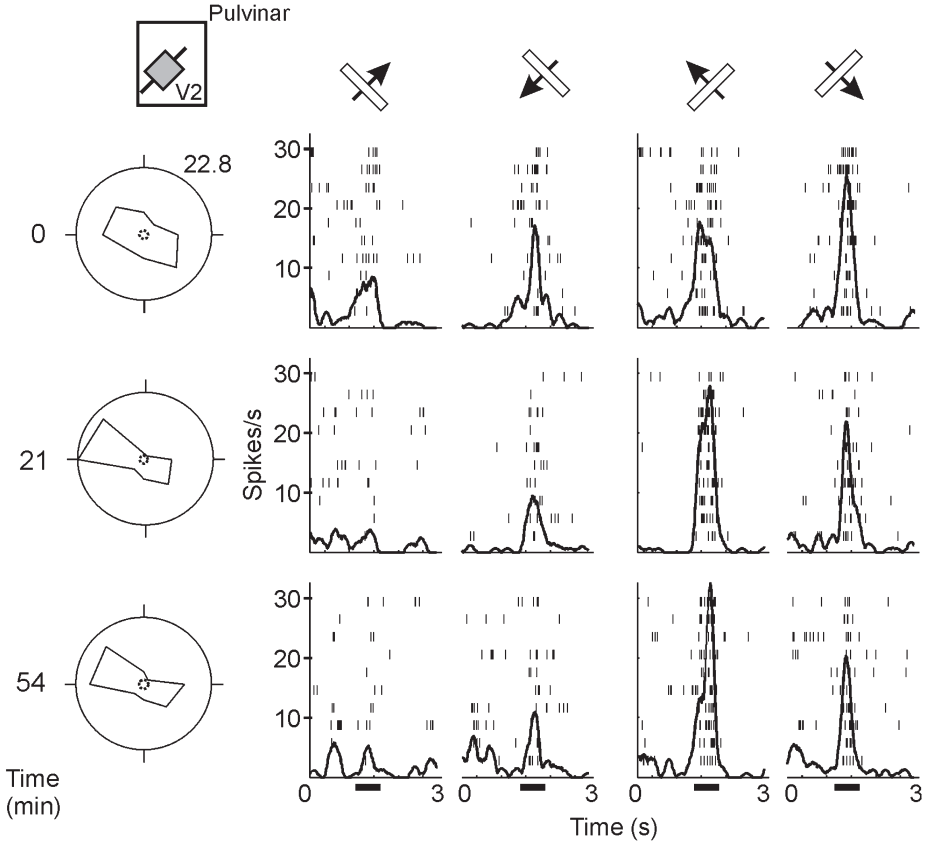

Fig. 9 Change in the preferred direction of movement in a V2 cell after GABA injection. Note the decrease in the cell's response to the preferred direction of movement (3158) and the increase in the response to the opposite direction with an increase in the directional index (DI). DI(0) = 0.17; DI(21) = 0.61. (See also legend for Fig. 4.)

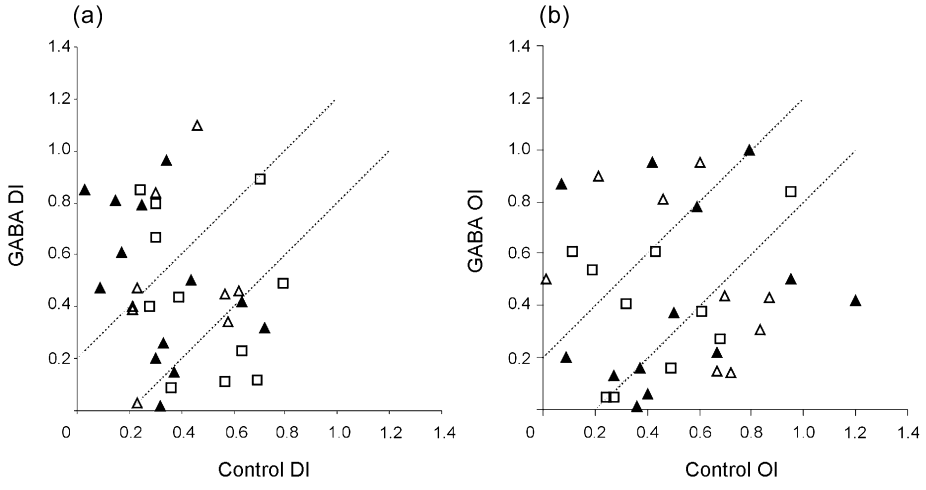

Fig. 10 Changes in direction and orientation selectivity of all cells studied in V2. (a) Correlation between the directional indices (DI) before (control DI) and after GABA injections (GABA DI). (b) Correlation between the orientation indices (OI) before (control OI) and after GABA injections (GABA OI). Broken lines with a slope of 1 represent the limits of significance of changes in indices (> 0.2). Changes in DI were observed in 66% of cells, whereas 72% showed changes in OI. (▲), excitatory modulation cells; (△), inhibitory modulation cells; (□), cells with no modulation.

The mechanisms responsible for orientation and/or directional selectivity in cortical cells are not yet well explained. Hubel and Wiesel proposed that the orientation selectivity was established by the organization of excitatory projections of the lateral geniculate nucleus.[37] Later studies showed the importance of cortical inhibitory and excitatory processes in the generation of this selectivity.[25,26,38,39] Sato *et al.* showed that administration of bicuculin in primary visual cortex of *Macaca* decreased the direction selectivity in most cells.[40] However, in most cases, an increased response remained in the original preferred direction. This suggests that the excitatory projections are the basis for the direction selectivity and that cortical inhibition seems to play a role in the determination of the strength of this directionality. However, our results show that, at least in area V2 of *Cebus* monkeys, in addition to the intracortical inhibitory mechanisms, projections originated from the pulvinar nucleus also play a modulatory role on the cell selectivity.

ACKNOWLEDGEMENTS

The authors are grateful to ES da Silva Filho, LH Pontes and MT Monteiro for technical assistance and to P Coutinho and G Coutinho for animal care. This research was supported by grants from PRONEX, CNPq, FAPERJ, FUJB.

REFERENCES

1. Rakic P. Embryonic development of the pulvinar–LP complex. In: Cooper IF, Riklan M, Rakic P (eds). *The Pulvinar–LP Complex.* Chas C Thomas, Springfield. 1974; 3–35.
2. Allman JM, Kaas JH, Lane RH, Miezin FM. A representation of the visual field in the inferior nucleus of the pulvinar in the owl monkey. *Brain Res.* 1972; **40**: 291–302.
3. Bender DB. Retinotopic organization of macaque pulvinar. *J. Neurophysiol.* 1981; **46**: 672–93.
4. Gattass R, Oswaldo-Cruz E, Sousa APB. Visuotopic organization of the *Cebus* pulvinar: A double representation of the contralateral hemifield. *Brain Res.* 1978; **152**: 1–16.
5. Adams MM, Webster MJ, Gattass R, Hof PR, Ungerleider LG. Visual cortical projections and chemoarchitecture of macaque monkey pulvinar. *J. Comp. Neurol.* 2000; **419**: 377–93.
6. Baleydier C, Morel A. Segregated thalamocortical pathways to inferior parietal and inferotemporal cortex in macaque monkey. *Vis. Neurosci.* 1992; **8**: 391–405.
7. Benevento LA, Davis B. Topographical projections of the prestriate cortex to the pulvinar nuclei in the macaque monkey: An autoradiographic study. *Exp. Brain Res.* 1977; **30**: 405–24.
8. Lin CS, Kaas JH. The inferior pulvinar complex in owl monkeys: Architectonic subdivisions and patterns of input from the superior colliculus and subdivisions of visual cortex. *J. Comp. Neurol.* 1979; **187**: 655–78.
9. Soares JGM, Gattass R, Souza APB, Rosa MGP, Fiorani Jr M, Brandão BL. Connectional and neurochemical subdivisions of the pulvinar in *Cebus* monkeys. *Vis. Neurosci.* 2001; **18**: 25–41.
10. Ungerleider LG, Galken TW, Mishkin M. Visuotopic organization of projections of striate cortex to inferior and lateral pulvinar in rhesus monkey. *J. Comp. Neurol.* 1983; **217**: 137–57.
11. Molotchnikoff S, Shumikhina S. The lateral posterior–pulvinar complex modulation of stimulus-dependent oscillations in the cat visual cortex. *Vis. Res.* 1996; **36**: 2037–46.
12. Rodman HR, Gross CG, Albright TD. Afferent basis of visual response properties in area MT of the macaque. I. Effects of striate cortex removal. *J. Neurosci.* 1989; **9**: 2033–50.
13. Rosa MGP, Tweedale R, Elston GN. Visual responses of neurons in the middle temporal area of New World monkeys after lesions of striate cortex. *J. Neurosci.* 2000; **20**: 5552–63.
14. Petersen SE, Robinson DL, Morris JD. Contributions of the pulvinar to visual spatial attention. *Neuropsychology* 1987; **25**: 97–105.
15. Rafal RD, Posner MI. Deficits in human visual spatial attention following thalamic lesions. *Proc. Nat. Acad. Sci. USA* 1987; **84**: 7349–53.
16. Laberge D, Buchsbaum MS. Positron emission tomographic measurements of pulvinar activity during an attention task. *J. Neurosci.* 1990; **10**: 613–19.
17. Rosa MGP, Souza APB, Gattass R. Representation of the visual field in the second visual area in the *Cebus* monkey. *J. Comp. Neurol.* 1988; **275**: 326–45.
18. Schiller PH, Malpeli JG. The effect of striate cortex cooling on area 18 cells in the monkey. *Brain Res.* 1977; **126**: 366–9.
19. Girard P, Bullier J. Visual activity in area V2 during reversible inactivation of area 17 in the macaque monkey. *J. Neurophysiol.* 1989; **62**: 1287–302.
20. Soares JGM, Diogo ACM, Fiorani M, Souza APB, Gattass R. Changes in orientation and direction selectivity of cells in secondary visual area (V2) after GABA inactivation of the pulvinar in *Cebus* monkeys. *Soc. Neurosci.* 2001; **27**: 620.12 (Abstract).
21. Gattass R, Gross CG. Visual topography of the striate projection zone in the posterior superior temporal sulcus (MT) of the macaque. *J. Neurophysiol.* 1981; **46**: 621–38.
22. Casanova C, Michaud Y, Morin C, McKinley PA, Molotchnikoff S. Visual responsiveness and direction selectivity of cells in area 18 during local reversible inactivation of area 17 in cats. *Vis. Neurosci.* 1992; **9**: 581–93.
23. Fiorani M, Azzi JC, Gattass R. Primate V1 has a visuotopic rather than a retinotopic organization: The topographic organization of the blind spot (BS) in V1. *Soc. Neurosci.* 2001; **27**: 619.46 (Abstract).
24. Crook JM, Kisvarday ZF, Eysel UT. GABA-induced inactivaction of functionally characterized sites in cat striate cortex: Effects on orientation tuning and direction selectivity. *Vis. Neurosci.* 1997; **14**: 141–58.
25. Crook JM, Kisvarday ZF, Eysel UT. GABA-induced inactivation of functionally characterized sites in cat visual cortex (area 18): Effects on direction selectivity. *J. Neurophysiol.* 1996; **75**: 2071–88.
26. Sillito AM, Kemp JA, Milson JA, Berardi N. A re-evaluation of the mechanisms underlying simple cell orientation selectivity. *Brain Res.* 1980; **194**: 517–20.
27. Hupé JM, Chouvet G, Bullier J. Spatial and temporal parameters of cortical inactivation by GABA. *J. Neurosci. Methods* 1999; **86**: 129–43.
28. Martin JH, Cooper SE, Ghez C. Differential effects of local inactivation within motor cortex and red nucleus on performance of an elbow task in the cat. *Exp. Brain Res.* 1993; **94**: 418–28.
29. de Mello FG. GABA-mediated control of glutamate decarboxylase (GAD) in cell aggregate culture of chick embryo retina. *Brain Res.* 1984; **316**: 7–13.
30. Fries P, Reynolds JH, Rorie AE, Desimone R. Modulation of oscillatory neuronal synchronization by selective visual attention. *Science* 2001; **291**: 1506–7.
31. Zeki S, Shipp S. Modular connections between areas V2 and V4 of macaque monkey visual cortex. *Eur. J. Neurosci.* 1989; **1**: 494–506.
32. Rockland KS, Pandya DN. Laminar origins and terminations of cortical connections of the occipital lobe in the rhesus monkey. *Brain Res.* 1979; **179**: 3–20.
33. Felleman DJ, Van Essen DC. Distributed hierarchical processing in the primate cerebral cortex. *Cerebral Cortex* 1991; **1**: 1–47.
34. Guillery RW, Sherman SM. Thalamic relay functions and their role in corticocortical communication: Generalizations from the visual system. 2002; 33: 163–75.
35. Levitt JB, Yoshioka T, Lund JS. Connections between the pulvinar complex and cytochrome oxidase-defined compartments in visual area V2 of macaque monkey. *Exp. Brain Res.* 1995; **104**: 419–30.

36. Horton JC, Sincich LC. Pale cytochrome oxidase V2 stripes receive the richest projection from macaque striate cortex. *Soc. Neurosci.* 2001; **27**: 11.3 (Abstract).
37. Hubel DH, Wiesel TN. Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *J. Physiol.* 1962; **160**: 106–54.
38. Hata Y, Tsumoto T, Sato H, Hagihara K, Tamura H. Inhibition contributes to orientation selectivity in visual cortex of cat. *Nature* 1988; **335**: 815–17.
39. Tsumoto T, Eckart W, Creutzfeldt OD. Modification of orientation sensitivity of cat visual cortex neurons by removal of GABA-mediated inhibition. *Exp. Brain Res.* 1979; **34**: 351–63.
40. Sato H, Katsuyama N, Tamura H, Hata Y, Tsumoto T. Mechanisms underlying direction selectivity of neurons in the primary visual cortex of the macaque. *J. Neurophysiol.* 1995; **74**: 1382–94.